

Table 1

BCR-ABL Intra Run
08/25/99

	K562 (10ng)		SUPB-15 (10ng)		K562 (10pg)		K562 (1pg)	
	Ct	Quantity	Ct	Quantity	Ct	Quantity	Ct	Quantity
1	20.91	18598	24.88	1414	32.30	11.50	36.66	0.68
2	21.09	16572	24.81	1477	32.15	12.68	36.67	0.68
3	21.02	17258	24.83	1461	32.67	9.06	36.69	0.67
4	21.06	16849	24.82	1476	32.44	10.54	36.32	0.85
5	20.89	18871	24.81	1485	32.35	11.13	36.39	0.81
6	21.12	16173	24.87	1429	32.53	9.93	37.94	0.30
7	21.07	16758	24.79	1498	32.46	10.35	35.44	1.50
8	21.04	17070	24.7	1589	32.19	12.34	36.55	0.7
9	20.89	18841	24.73	1559	32.80	8.31	36.99	0.55
10	21.03	17219	24.89	1410	32.59	9.56	36.6	0.71
11	20.91	18534	25.05	1266	32.44	10.53	34.82	2.25
12	20.95	18143	24.83	1460	32.62	9.36	35.85	1.15
Average	21.00	17573.57	24.83	1460.17	32.46	10.44	36.41	0.91
SD	0.08	963.80	0.09	81.05	0.19	1.31	0.78	0.52
%CV	0.40	5.48	0.35	5.55	0.60	12.55	2.15	57.14

Reaction volume is 30ul.

Table 2

BCR-ABL Inter-Run
09/02/99

	Date	K562 (10ng)		SUPB-15 (10ng)		STD 10 ⁵ ng	Threshold
		Ct	Quantity	Ct	Quantity	Ct	
1	08/10/99	21.02	10809	24.22	1309	17.73	0.030
2	08/11/99	19.90	22791	23.63	2049	17.72	0.027
3	08/12/99	19.89	21106	23.61	1823	17.70	0.025
4	08/16/99	20.10	17892	23.70	1493	17.78	0.022
5	08/17/99	20.3	18781	23.96	1520	17.71	0.029
6	08/23/99	20.39	16744	24.39	1539	17.72	0.033
7	08/25/99	20.14	19311	24.19	1368	17.73	0.028
Average		20.22	18204.84	23.95	1585.84	17.73	
SD		0.18	1629.89	0.33	167.27	0.03	
%CV		0.88	8.95	1.36	10.55	0.17	

Table 3

BCR-ABL TaqMan RT-PCR (BCR-ABL071299)

P1, P2 and P3 (Threshold @ 0.020)

Sample	Ct			Quantity (pg)			Translocation	
	# 1	# 2	Average	# 1	# 2	Average	TaqMan	Dot Blot
K562 Spk 10^6 (0.01ml)	21.15	20.77	20.96	52613	65768	59190	Pos	
K562 Spk 10^5 (0.02ml)	17.34	17.56	17.45	515059	450642	482850	Pos	
K562 Spk 10^4 (0.02ml)	21.08	20.87	20.98	54604	62021	58312	Pos	
K562 Spk 10^3 (0.02ml)	23.64	23.92	23.78	11774	9951	10862	Pos	
K562 Spk 10^2 (0.02ml)	28.45	28.28	28.37	658	729	694	Pos	
K562 Spk 10 (0.02ml)	32.84	31.01	31.93	47	142	95	Pos	
Normal (2.0+0) (0.02ml)	45.00	45.00	45.00	0	0	0	Neg	
Normal (1.0+1.0)(0.02ml)	45.00	39.38	42.19	0	1	0	Neg	
K562 Spk 10^6 (100ng)	16.29	16.33	16.31	966643	942784	954713	Pos	
K562 Spk 10^5 (100ng)	17.95	17.77	17.86	357147	398169	377658	Pos	
K562 Spk 10^4 (100ng)	21.11	21.11	21.11	53909	53651	53780	Pos	
K562 Spk 10^3 (100ng)	23.31	23.14	23.23	14339	15884	15112	Pos	
K562 Spk 10^2 (100ng)	28.60	28.70	28.65	602	565	584	Pos	
K562 Spk 10 (100ng)	30.29	30.36	30.33	218	209	214	Pos	
Normal (2.0+0) (100ng)	34.52	35.41	34.97	17	10	14	Neg?	
Normal (1.0+1.0)(100ng)	45.00	45.00	45.00	0	0	0	Neg	

Table 4

Clinical Samples

	Sample	Ct			Quantity (pg)			cDNA (ng)			Ass Date
		#1	#2	Ave.	#1	#2	Average		TaqMan	Dot Blot	
1	BC9-266	28.02	28.31	28.17	123.6	101.4	112.5	50	Pos	P3	07/12/99
2	BC9-267	37.94	40.00	38.97	0.2	0.0	0.1	50	Not Detec	Neg	07/12/99
3	BC9-268	40.00	40.00	40.00	0.0	0.0	0.0	50	Not Detec	Neg	07/12/99
4	BC9-269	20.80	21.28	21.04	11614.1	16087.6	13850.9	50	Pos	P2, P3	07/12/99
5	BC9-270*	40.00	40.00	40.00	0.0	0.0	0.0	50	V W Amp	Neg	07/12/99
6	BC9-271	20.71	20.92	20.82	17070.9	14843.8	15957.3	50	Pos	P2, P3	07/12/99
7	BC9-272	35.01	34.14	34.58	1.1	2.0	1.6	50	Not Detec	Neg	07/12/99
8	BC9-273	40.00	32.86	36.43	0.0	4.7	2.4	50	Not Detec	Neg	07/12/99
9	BC9-274	26.78	30.84	28.81	285.2	18.5	151.9	50	Pos	P3	07/12/99
10	BC9-514BM*	5.00	45.00	45.00	0.0	1.1	0.6	100	W Amp	Neg	08/10/99
11	BC9-515BM	20.44	21.46	20.95	14131.9	7247.9	10689.9	100	Pos	P2	08/10/99
12	BC9-516	24.28	24.24	24.26	1128.7	1156.8	1142.8	100	Pos	P3	08/10/99
13	BC9-540	21.37	22.22	21.80	3836.5	3324.4	3580.5	100	Pos	P1	08/11/99
14	BC9-571	45.00	43.49	44.25	0.0	0.0	0.0	100	Not Detec	Neg	08/11/99
15	BC9-572	45.00	43.01	44.01	0	0	0	60	Not Detec	Neg	08/12/99
16	BC9-573	42.05	41.79	41.92	0	0	0	51	Not Detec	Neg	08/12/99
17	BC9-574	40.54	45.00	42.77	0	0	0	50	Not Detec	Neg	08/12/99
18	BC9-575A	45.00	45.00	45.00	0	0	0	100	Not Detec	Neg	08/12/99
19	BC9-576	45.00	43.75	44.38	0	0	0	100	Not Detec	Neg	08/12/99
20	BC9-275	24.95	24.14	24.55	549.6	957.6	753.6	100	Pos	P3	08/16/99
21	BC9-276	39.28	36.46	37.87	0.0	0.2	0.1	50	Not Detec	P2	08/16/99
22	BC9-277	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Neg	08/16/99
23	BC9-278	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Neg	08/16/99
24	BC9-279	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Neg	08/16/99
25	BC9-280	45.00	45.00	45.00	0.0	0.0	0.0	36	Not Detec	Neg	08/16/99
26	BC9-275	23.73	23.87	23.80	1222.0	1115.3	1168.6	50	Pos	P3	08/17/99
27	BC9-272	45.00	45.00	45.00	0.1	0.0	0.1	50	Not Detec	Neg	08/23/99
28	BC9-273	45.00	45.00	45.00	0.1	0.1	0.1	50	Not Detec	Neg	08/23/99
29	BC9-517	36.15	36.11	36.13	1.2	1.2	1.2	50	Not Detec	Neg	08/23/99
30	BC9-518	34.88	35.56	35.22	1.7	2.7	2.2	50	Not Detec	Neg	08/23/99
31	BC9-519	36.30	35.15	35.73	1.1	1.2	1.1	50	Not Detec	Neg	08/23/99
32	BC9-520	35.52	36.03	35.78	1.8	1.3	1.5	50	Not Detec	Neg	08/23/99
33	BC9-522	37.33	38.06	37.70	0.5	0.3	0.4	50	Not Detec	Neg	08/23/99
34	BC9-296	28.76	29.30	29.03	114.1	80.5	97.3	50	Pos	P2, P3	08/25/99
35	BC9-297*	45.00	45.00	45.00	13.9	0.0	6.9	50	W Amp	Neg	08/25/99
36	BC9-298	23.87	24.12	24.00	2727.0	2320.1	2523.5	50	Pos	P2, P3	08/25/99
37	BC9-540	22.10	22.01	22.06	8614.0	9091.2	8852.6	50	Pos	P1	08/25/99

* indicates that the sample's internal control was weakly amplified (W Amp), or very weakly amplified (V W Amp).

Cut-off for positive is 10 pg.

* Positive on dot blot, negative on TaqMan PCR.

Total 34 clinical samples.

Table 5

BCR-ABL Validation Studies

Normal											
	Sample	Ct			Quantity (pg)			cDNA	TaqMan		Ass Date
		#1	#2	Ave.	#1	#2	Average	(ng)			
1	14-1 (TRIZOL)	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Normal	08/10/99
2	14-1 (RNA S	39.61	45.00	42.31	0.1	0.0	0.0	100	Not Detec	Normal	08/10/99
3	10-1	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Normal	08/10/99
4	33-1	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Normal	08/10/99
5	147-1	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Normal	08/10/99
6	199-1	41.58	45.00	43.29	0.0	0.0	0.0	100	Not Detec	Normal	08/10/99
7	250-1	36.65	45.00	40.83	0.3	0.0	0.2	100	Not Detec	Normal	08/10/99
8	448-1	45.00	35.51	40.26	0.0	0.7	0.3	100	Not Detec	Normal	08/10/99
9	607-1	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Normal	08/10/99
10	608-1	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Normal	08/10/99
11	159-1	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Normal	08/11/99
12	348-1	45.00	36.20	40.60	0.0	0.3	0.2	100	Not Detec	Normal	08/11/99
13	384-1	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Normal	08/11/99
14	394-1	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Normal	08/11/99
15	410-1	45.00	45.00	45.00	0.3	0.0	0.2	100	Not Detec	Normal	08/11/99
16	432-1	45.00	37.30	41.15	0.0	0.2	0.1	100	Not Detec	Normal	08/11/99
17	445-1	45.00	42.69	43.85	0.0	0.0	0.0	100	Not Detec	Normal	08/11/99
18	610-1	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Normal	08/11/99
19	86-1	42.98	45.00	43.99	0.0	0.0	0.0	100	Not Detec	Normal	08/12/99
20	105-1	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Normal	08/12/99
21	194-1	29.67	42.95	36.31	34.0	0.0	17.0	100	?	Normal	08/12/99
22	285-1	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Normal	08/12/99
23	294-1	45.00	43.34	44.17	0.0	0.0	0.0	100	Not Detec	Normal	08/12/99
24	594-1	45.00	45.00	45.00	0.0	0.0	0.0	100	Not Detec	Normal	08/12/99
25	194-1	45.00	36.71	40.86	0.0	0.8	0.4	50	Not Detec	Normal	08/23/99

24 normals if 1 + 2 counts as different samples -